Table 1 shows that D-Di values were not statistically different between groups I, II and healthy subjects without thrombotic antecedents. Two patients in group I had D-Di above the critical cut-off of 500 ng/ml. One displayed a borderline value at 540 ng/ml. The other had had several episodes of DVT, the latest 6 months before, at the age of 67 years. Vena cava interruption was performed on this occasion. Her D-Di value was 850 ng/ml. It has been demonstrated that normal values of D-Di increase with age (1). Critical cut-off is probably higher in the elderly and this value might be considered as normal for the patient's age. Alternatively, this might be an effect of the vena cava filter, although another patient with a filter in group I displayed a low D-Di value.

We previously found that, 3 months after a DVT, D-Di levels returned to the normal range established in an age-matched control population, when the patients were correctly treated by oral anticoagulants (2). Although that study and the present one were not performed in the same patients, these results suggest that once D-Di level has returned to its baseline value, within 3 months after the acute episode, it remains stable and low after the anticoagulant treatment is stopped.

Measurement of D-Di is therefore of potential value for the diagnosis of recurrent DVT. This conclusion is restricted to the patient population of this study, i.e. young patients without symptoms of post-phlebitic syndrome, associated disease, or inherited disorder predisposing to thrombosis.

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High Titer Inhibitors in Severe Haemophilia A

A Meta-analysis Based on Eight Long-term Follow-up Studies concerning Inhibitors Associated with Crude or Intermediate Purity Factor VIII Products

Sir,

The recent introduction of factor VIII products produced by recombinant DNA technology for the treatment of patients with hemophilia A has rekindled the interest in the occurrence of anti-factor VIII antibodies (1, 2). Specific patient characteristics may be associated with a high risk of inhibitor formation, e.g., gene deletions. Additionally, some products cause more inhibitors than others, possibly due to neoantigens induced by the production process (3, 4). Unfortunately, there is insufficient information in the literature that might be used as a historical yardstick for the comparison of inhibitor incidences associated with older generations of products. Plasma derived factor VIII, purified using monoclonal antibodies, could serve as a suitable comparison for the recombinant products but the two published prospective studies on these products are very small and were not specifically designed for inhibitor surveillance (5, 6). New studies on these products in previously untreated patients are ongoing. There are eight long-term follow-up studies from single haemophilia centers or groups of centers that can provide useful information on crude and intermediate purity products (7–14). In each of these studies, all patients were followed from birth by inhibitor measurements at regular outpatient visits. However, their comparison is hampered by differences in the study designs.

The authors of the eight studies have collaborated to produce one set of consistent data and to provide useful information on the risk of high response type inhibitors in patients with severe haemophilia. By restricting the analysis to severe haemophilia (defined as <1% factor VIII in all studies) we focussed on those patients with the highest risk. In this way we avoided the inclusion of patients with very little exposure to factor VIII, since virtually all patients with severe haemophilia have had a sizable numer of exposures by the age of five years. Unfortunately, we could not use the number of exposure days for the time axis, since this information was not available for several of the studies. The restriction to high response type inhibitors served to concentrate on inhibitors that are clinically significant. For three studies "high response" was defined as a maximum inhibitor titer in excess of ten Bethesda units (8, 10, 11) and in five it was defined as in excess of five Bethesda units (7, 9, 12–14). The authors combined the raw data from their studies and this allowed us to calculate a cumulative incidence for the total group of 451 patients. The cumulative incidence curves for the individual studies are shown in Fig. 1a and the curve for the combined set of data in Fig. 1b. At the age of three years the cumulative incidence was 10 percent (95 percent confidence interval: 7 to 13 percent) and this gradually increased to 20 percent at age 18.

Although we eliminated two major differences by restricting the analysis to severe haemophilia and high response type inhibitors it is clear that some differences remain that may have contributed to the widely varying results. First, transiency of the inhibitors was reported rather frequently for studies 3 (9) and 8 (14), which may have led to higher incidences than in the other studies. Second, in study 7 (13) only cryoprecipitate was used and similarly, all inhibitors found in study 2 (8) occurred during the period that all patients exclusively used cryoprecipitate. Apparently, the very low inhibitor incidence in study 7 cannot be ascribed solely to the use of this crude product. Third, the in-

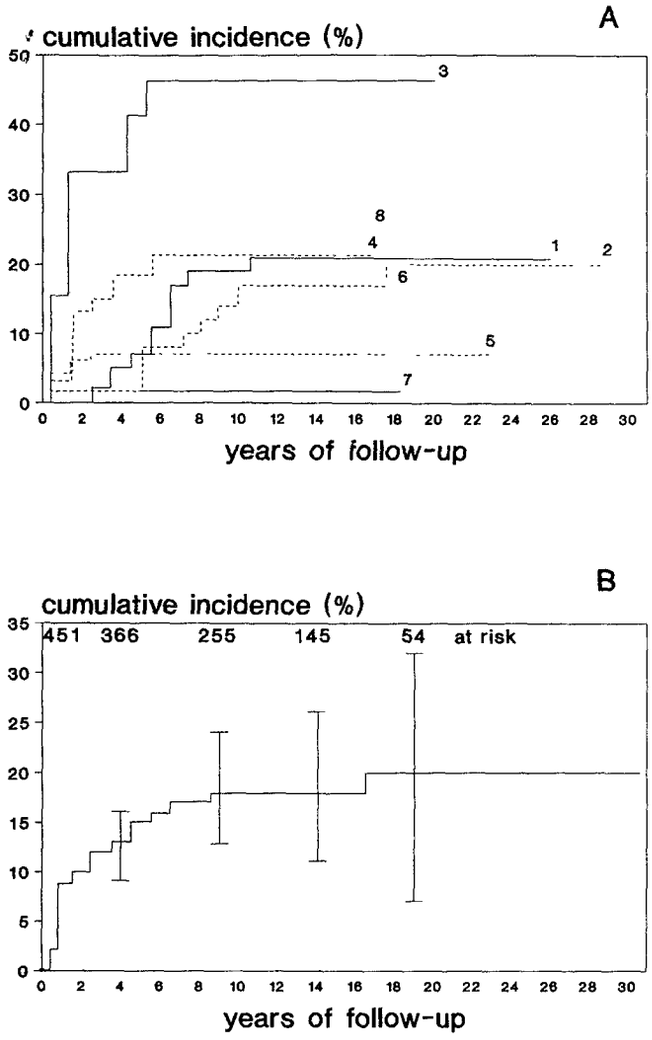

Fig. 1 The cumulative incidence with 95% confidence intervals of high response type factor VIII inhibitors in severe haemophilia A based on the data from the eight follow-up studies (individual data panel A, combined data panel B). Study 1 = ref 7, study 2 = ref 8, study 3 = ref 9, study 4 = ref 10, study 5 = ref 11, study 6 = ref 12, study 7 = ref 13, study 8 = ref 14

tensity of factor VIII replacement was different. In Table 1, the intensity of replacement is shown next to the height of the plateau of the cumulative incidence curves. It appears that the intensity of replacement is not an exclusive determinant of the inhibitor incidence. Finally, except for studies 7 and 2, most patients from most centers used more than one product. If neo-antigens in the products play a role, every switch from one product to another may be associated with additional risk for inhibitor formation.

The findings of this study show that one needs many years of exposure before a plateau is reached. Consequently, prospective studies with new products require long follow-up. In addition, the wide confidence interval around the curve for the combined data suggests that it will require prohibitively large numbers of patients to prove that a new product is safer or less safe than the older products. For this reason we have proposed (15) that national or international pharmacovigilance programmes be undertaken for this purpose. Furthermore, for future studies we recommend to do separate analyses for patients with haemophilia of different severities, to use exposure days for the time axis and to concentrate on clinically relevant inhibitors. Consensus should be sought for the definition of "clinically relevant". As a minimum this

Table 1 The cumulative inhibitor incidence at the plateau phase related to the intensity of factor VIII replacement*

Study number	Cumulative incidence plateau (%)	Annual factor VIII dose U/kg/year
7	2	1400 – 2000
5	7	>2000
6	19	200 – 800
2	20	200 – 800
1	21	200 – 800
4	21	200 – 800
8	25	200 – 800
3	46	800 – 1400

*The authors were asked to indicate their usual annual factor VIII dose per kg bodyweight: <200, 200 – 800, 800 – 1400, 1400 – 2000, or >2000 Units per kg per year.

term should imply criteria for inhibitor titer and persistence of the inhibitor, but possibly other aspects as well.

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Endothelial Cell Markers (vWF, t-PA and PAI-1) in the Elderly

Dear Sir,

Within the frame of a study intended to determine fibrinolytic parameters in an elder population, we have studied a group of 47 patients strictly comparable for age and sex ratio to a control group of 26 subjects. Various disease processes commonly found in the elderly were documented in the group of patients (Table 1). Control of elder subjects had no evolutive pathology as suggested by a normal clinical examination, C reactive protein (CRP) levels below 10 g/l and normal coagulation routine tests. The parameters studied were compared to those obtained in a younger control group (<60 years old, n = 20).

In a preliminary communication (1) we reported the levels of prourokinase (scu-PA) antigen and activity levels in a geriatric population. Scu-PA antigen was quantified with a double sandwich ELISA, as described (2); scu-PA activity was measured using a Bio Immuno Assay in which the scu-PA bound to a first antibody was activated with plasmin and then colorimetrically measured (3). No significant differences were found between controls and patients (Table 1) nor between our geriatric healthy population and the younger control group (3.6 ± 0.9 ng/ml, mean ± SD versus 3.5 ± 1.1 ng/ml for scu-PA antigen; 2.36 ± 0.6 ng/ml versus 2.1 ± 0.4 ng/ml for scu-PA activity). However, in five patients (acute myeloid leukemia, n = 1; severe liver failure, n = 2; and metastatic neoplasia, n = 3), an abnormal high level of scu-PA antigen and activity was documented.

This work has been presently extended to the determination of von Willebrand factor (vWF), tissue plasminogen activator (t-PA), and its inhibitor (PAI-1) measured with ELISA assays (respectively Asserachrom vWF, t-PA and PAI from Diagnostica Stago, Asnières, France). Acute phase reactant proteins (fibrinogen, CRP) were also determined in both geriatric subjects and in a younger control group (<60 years).

A statistical significant difference (p <0.01, Mann and Whitney test) between elder controls and patients for CRP and fibrinogen levels (Table 1) was suggestive of a chronic inflammatory state in the patient group; patients also had increased vWF levels (1.97 ± 0.84 u/ml versus 1.62 ± 0.59 u/ml for controls, p = 0.03) which were strongly correlated with CRP (p <0.01). As previously reported by other groups (4), vWF levels were higher in the elderly (vWF antigen level for the younger control group was 1.0 ± 0.33 u/ml, mean ± SD).

The levels of t-PA and PAI-1 were comparable in the elder healthy and ill groups (Table 1). In agreement with a previous report (5), t-PA levels were higher in the geriatric population (13 ± 9.7 ng/ml, mean ± SD) as compared to those (5.5 ± 3 ng/ml) of a younger control group (<60 years). A concomitant increase in PAI-1 (35.7 ± 26 ng/ml versus 16.4 ± 11.9 ng/ml, mean ± SD for the younger healthy group) was observed and was shown to be significantly correlated with t-PA levels (p = 0.01) and to the CRP, i.e., the inflammatory state of the patients (p = 0.01).

In control older subjects, proteins synthetized by the vascular endothelium, vWF and PAI-1, were shown to be increased whereas CRP and fibrinogen, synthetized by the liver remained within the normal range, thus suggesting chronic stimulation of the endothelium. The well known increase in the plasma level of t-PA antigen is probably related to the same phenomenon although a decrease in the hepatic clearance of t-PA may also be evoked.

Finally, no correlation was found between the levels of scu-PA and the following parameters: CRP, vWF antigen, t-PA and PAI-1 antigens and serum creatinine level.

Table 1

	N ratio M/F	Age (years) mean ± SD	scu-PA Ag (ng/ml) mean ± SD (range)	t-PA Ag (ng/ml) median (range)	PAI-1 Ag (ng/ml) median (range)	CRP (g/l) median (range)	vWF Ag (u/ml) mean ± SD	Fibrinogen (g/l) mean ± SD
Controls	26 (7/19)	81 ± 8	3.6 ± 0.9 (2.2 – 5.8)	11.8 (3.8 – 46)	27.5 (9.5 – 100)	5 (5 – 10)	1.62 ± 0.59	3.7 ± 0.9
Patients ‡	47 (13/34)	82 ± 7	5.4 ± 8.1 (1.6 – 56.1)	10 (3.5 – 46)	24 (6.5 – 120)	13 (5 – 246)	1.97 ± 0.84	4.6 ± 1.6
p value*			NS	NS	NS	<0.01	<0.05	<0.01

NS: non significant
*: Mann-Whitney test
‡: with the following pathology: iron, folic acid and vitamin B12 deficiencies (n = 7), chronic lymphocytic leukemia and lymphoma (n = 5), acute myeloid leukemia (n = 1), cerebral or peripheral vascular ischaemia (n = 9), digestive tract neoplasia (n = 9), diabetes (n = 1), various inflammatory reactions (n = 3), bacterial infections (n = 2) and renal (n = 5), pulmonary (n = 3) or liver (n = 2) disease.